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- (71) Applicant (for all designated States except US): DSM IP ASSETS B.V. [NL/NL]; Het Overloon 1, NL-6411 Te Heerlen (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): STREEKSTRA, Hugo [NL/NL]; Weteringstraat 28-1, NL-1017 SP Amsterdam (NL). BROCKEN, Petrus, Joseph, Maria [NL/NL]; Zwaluw 2, NL-2678 XC de Lier (NL).
- (74) Agents: WRIGHT, Simon, Mark et al.; J.A. Kemp & Co, 14 South Square, Gray's Inn, London WC1R 5JJ (GB).

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(54) Title: PREPARATION OF MICROBIAL OIL CONTAINING POLYUNSATURATED FATTY ACIDS

(57) Abstract: The present invention provides a process for the production of a microbial oil comprising culturing a micro-organism in a two stage fermentation process where, in a last stage that precedes the end of fermentation, the carbon source is: consumed by the micro-organisms at a rate greater than it is added to the medium; added at a rate ≤ 0.30 M carbon/kg medium; or is rate limiting on the growth of the micro-organism. The micro-organisms thus have the carbon source restricted so that they preferentially metabolise fats or lipids other than arachidonic acid (ARA), so increasing the proportion of ARA in the cells. A microbial oil is then recovered from the micro-organism, using hexane as a solvent, that has at least 50% ARA and at least 90% triglycerides.

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A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12P7/64 C11C1/00 IPC 7 A23L1/30 A61K7/00 A61K7/48 A61K31/20 A61K31/232 //(C12P7/64,C12R1:645) According to International Patent Classification (IPC) or to both national classification and IPC Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12P C11C A23L A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, BIOSIS, WPI Data, MEDLINE, EMBASE, PAJ C. DOCUMENTS CONSIDERED TO BE RELEVANT Category ⁴ Citation of document, with Indication, where appropriate, of the relevant passages Relevant to claim No. Α EP 1 035 211 A (SUNTORY LTD) 1-6 13 September 2000 (2000-09-13) cited in the application claims 1-6; examples 1,4-6; table 3 X claims 11,12,17-20 7 Α WO 96 21037 A (MARTEK BIOSCIENCES CORP 1-11 ;KYLE DAVID J (US)) 11 July 1996 (1996-07-11) cited in the application the whole document Further documents are listed in the continuation of box C. Patent family members are listed in annex. Special categories of cited documents: 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance 'E' earlier document but published on or after the international *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claillon or other special reason (as specified) "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu-'O' document referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled in the art. document published prior to the international filing date but later than the priority date claimed *&* document member of the same patent family Date of the actual completion of the international search Date of mailing of the International search report 18 February 2004 09/03/2004 Name and mailing address of the ISA Authorized officer European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 Döpfer, K-P

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